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Unusual Hepatitis B Surface Antigen Variation in a Child Immunised Against Hepatitis B

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Perinatal transmission of and infection with hepatitis B (HBV) in early childhood are observed in a small proportion of the offspring of hepatitis B surface antigen (HBsAg)–positive mothers who are vaccinated against HBV immediately after giving birth. The children may be infected by wild-type HBV or by variants with amino acid substitutions in the "a" determinant of HBsAg, particularly at position 145 and, rarely, at positions 120, 126, 129, 131, 141, and 144. Four hundred and forty-six newborn infants of HBsAg-positive mothers in the northeastern part of the Czech Republic received combined active and passive immunisation against HBV. Only one child became an HBsAg carrier. This followed a mild, acute HBV illness in the beginning of the second year of his life. HBV DNA encoding the "a" determinant and surrounding region of HBsAg was sequenced after amplification from the plasma of the child and his mother. The child was infected with variants of HBsAg with substitutions at residues 137 and 139. The virus of the mother had changes at residues 120 and 121. HBV from both child and mother had an unusual substitution at residue 118 and seemed to be of the *ayw* subdeterminant. ***J. Med. Virol. 61:11–14, 2000.***

KEY WORDS: antibody escape variant; newborns; perinatal transmission; Czech Republic

INTRODUCTION

Combined active and passive immunisation of newborns against hepatitis B virus (HBV) is highly effective in preventing maternal transmission of HBV and in the development of the carrier state. Nevertheless, perinatal transmission and infection in early childhood are observed in a small percentage of immunised children, who may be infected by wild-type virus or by variants with amino acid substitutions in the "a" determinant of hepatitis B surface antigen (HBsAg). Such variants may not be neutralised by vaccine-induced antibodies [Waters et al., 1992]. The mutation reported most commonly in immunised children causes a substitution of arginine for glycine at position 145 of HBsAg [Carman et al., 1990; Harrison et al., 1991; Oon et al., 1995, 1996]. Other infections of children with vaccine-induced antibodies have been associated with substitutions at positions 120, 126, 129, 133, 141, and 144 [Harrison et al., 1994, 1996; Karthigesu et al., 1994; Ngui et al., 1997].

A programme of passive–active immunisation of the neonates of HBsAg-positive mothers was initiated in the Czech Republic in 1988–1989. Immunisations usually were carried out by paediatricians, and groups of children were followed in only a few regions. Observation of the largest of these groups, 446 vaccinated children, continues in the town of Ostrava, in the northeastern part of the Czech Republic. Evidence of perinatal transmission of HBV was sought in this group of infants. Other risk groups in the Czech Republic also are immunised against HBV. Vaccination of some health staff (e.g., personnel in dialysis units, in-

Clinical observation of the vaccinated child and his mother took place at the Department of Infectious Diseases, University Hospital in Ostrava, Czech Republic (L.R., I.O.). Hepatitis B virus DNA from the child and his mother was sequenced in the Department of Medicine, Royal Free and University College Medical School, University College London (T.J.H., Z.-L.F., R.L.). Serologic investigations were performed at the Institute of Hygiene, Ostrava, Czech Republic (I.L.), and polymerase chain reaction diagnostics at the Department of Clinical Biochemistry in Hradec Kralove, Czech Republic (L.P.).

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fectious disease departments, and other specialities) commenced in 1982. Since 1987, all students of medicine and persons enrolled in health schools are immunised. Vaccination of patients in dialysis units and patients with serious renal dysfunction commenced in 1988. Over the past 10 years, the incidence of acute HBV and the prevalence of chronic HBV have declined in the Czech Republic. At present, the seroprevalence of HBsAg is estimated at 0.5–0.7%, and evidence of past exposure (anti-HBV core antigen positivity) is found in 6–7%. A policy of universal immunisation of infants and older children has not yet been implemented in the Czech Republic.

PATIENTS AND METHODS

Immunisation of Infants

Passive–active immunisation of the neonates of HBsAg-positive mothers commenced in the Department of Infectious Diseases of the University Hospital in Ostrava in 1988. The number of children immunised annually increased gradually. At the end of 1998, this group included 446 newborns (433 Caucasian and 13 ethnic Chinese) from the northeastern region of the Czech Republic; 421 of these children are now more than 12 months old. All mothers were HBsAg positive during pregnancy, and 24 were also hepatitis B e antigen (HBeAg) positive.

Newborns were vaccinated using three immunisation schedules. The children received 50 or 100 IU of hepatitis B immune globulin (HBIG) intramuscularly (Hepaga, Sevac, Czech Republic) or, since 1995, intravenously (Hepatect; Biotest Pharma, Germany) within 12–24 hours after birth. Most of the children received their first dose of vaccine within 24 hours after birth. The children received 10-μg doses of plasma-derived vaccine (H-B-Vax; Merck Sharp & Dohme, Rahway, NY) or, from 1990, recombinant vaccine (Engerix B; SmithKline Beecham, Belgium), using the standard schedule of 0, 1, and 6 months for the majority. Twelve children of HBeAg-positive mothers were immunised at intervals of 0, 1, and 2 months. Only 11 children, who were at risk of tuberculosis, were immunised according to a third schedule. These infants received three doses of HBIG during their first 3 months of life. Active immunisation was started later; three doses of vaccine were administered at 3, 4, and 9 months of age. The immunisation schedules were completed in 412 children.

Serum Samples

Serum samples were obtained at birth (umbilical cord and venous blood), 1–3 months after completion of the immunisation schedule, at 2 years of age, and biennially thereafter. Samples were tested by enzyme-linked immunosorbent assay for HBsAg and anti-HBs (commercially available kits: Sevac and, since 1994, Sorin Biomedica, Italy) and for HBeAg, anti-HBc, anti-HBe, and anti-HBc IgM antibodies (commercial kits; Sorin Biomedica) in the Department of Immunology, Institute of Hygiene in Ostrava.

Starting in 1996, the presence of HBV DNA was determined using the polymerase chain reaction (PCR) in the Department of Clinical Biochemistry, University Hospital, Hradec Kralove, Czech Republic. The PCR targeted the pre-core region of HBV. The sensitivity of PCR was 10^5 genomes of HBV DNA per millilitre in 1996. Nested PCR was used beginning in 1997; this method increased the sensitivity to 10^2–10^3 genomes of HBV DNA per millilter. All determinations were performed in duplicate. The test was repeated from the same serum sample if the first result was positive or equivocal. HBV DNA encoding the "a" determinant and surrounding region of HBsAg from one child and his mother was sequenced in the Department of Medicine, Royal Free and University College Medical School, England, using published methods [Harrison et al., 1994].

RESULTS AND DISCUSSION

Investigation of Cord Blood

According to previous immunisation policies of the Czech Republic, HBsAg positivity of umbilical cord blood was an indication for discontinuing vaccination against HBV. However, maternal HBsAg may contaminate cord blood, and its presence does not predict the outcome of perinatal infection [Lee et al., 1978]. Accordingly, the official Czech recommendations were not followed, and immunisation of such infants was undertaken along with a detailed investigation of the cord blood [Roznovsky and Lochman, 1995].

The umbilical cord blood of 401 neonates was tested for HBsAg. HBsAg was detected in 141 samples, 203 samples showed negative results. The remaining 57 samples gave equivocal results, falling into the grey zone (the grey zone was determined to be in the range of 0.9–1.2 of cutoff for the assays). Most of the infants whose cord blood was positive for HBsAg did not have detectable antigen in venous blood, and no child was HBsAg positive at the end of the first month. Commencing in 1993, anti-HBc IgM was sought in the cord blood of 75 infants; all showed negative results. From 1996, the cord blood of 33 newborns was tested for HBV DNA using PCR; 14 samples were positive for HBsAg, but none was unequivocally positive by PCR (two nested PCR results from children with HBsAg in the cord blood were interpreted as equivocal). The presence of HBsAg in approximately one-third of the newborns, and the two equivocal nested PCR tests, may be attributed to contamination with maternal blood. In accordance with these findings, the immunisation protocol in the Czech Republic was changed in 1998. Neonates now are not tested routinely for HBsAg, and antigenaemia is not a reason for discontinuing immunisation.

Presence of HBsAg After Vaccination

The immunisation schedules were completed in 412 of 446 children. Of these children, 402 were tested after immunisation, most of them repeatedly. Protective titres of anti-HBs were produced by most children, and only one child became an HBsAg carrier. Of the 402 children, 316 were tested for anti-HBs antibodies 1–3

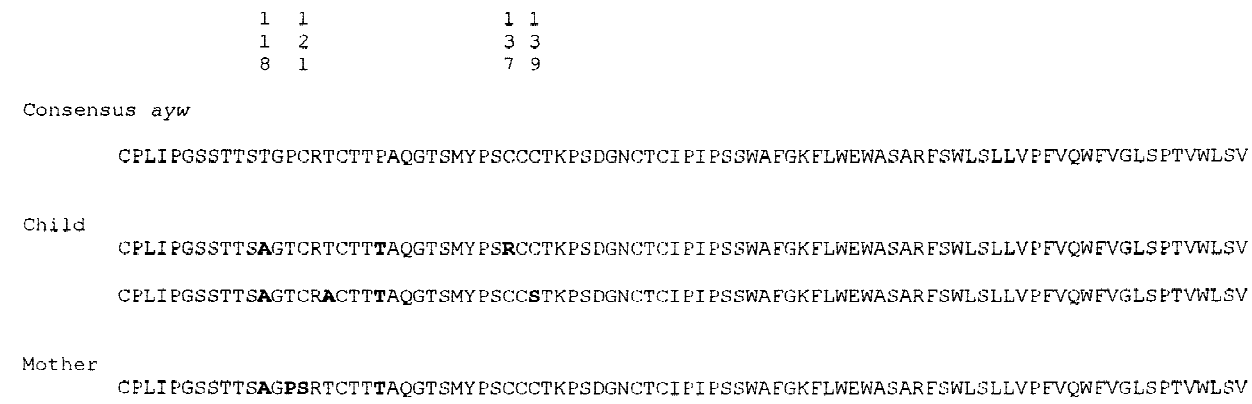

Fig. 1. Amino acid sequences of the "a" determinant and surrounding region of HBsAg predicted from HBV DNA amplified and cloned from the serum samples of the infected child (two variants) and his mother. The sequence of the equivalent region of subtype *ayw* HBV from the Genbank and EMBL databases is given above.

months after completion of the immunisation schedule, when the anti-HBs response was expected to be the highest. Anti-HBs levels above 50 mIU/ml were detected in 143 of 145 children (98.6 %) tested using the Sevac kit (a competitive enzyme immunoassay of lower sensitivity that was used until 1994), and anti-HBs above 10 mIU/ml was detected in 161 of 171 children (94.2 %) tested thereafter using commercial reagents (Sorin Biomedica).

Only one (ethnic Caucasian) child, whose mother was HBsAg and HBeAg positive, became persistently infected with HBV. The child was delivered vaginally and breast-fed for only 1 month. His cord blood tested equivocally for HbsAg, but the antigen was not detected in venous blood in the first month after birth. The child received 50 IU of HBIG (Hepaga, prepared from the plasma of donors who had recovered naturally from HBV and supplemented with anti-HBs from donors immunised with plasma-derived or recombinant vaccines). He was immunised with recombinant vaccine (Engerix B) according to the standard schedule, which was completed at the 8th month. The first serologic investigation after immunisation was carried out at 11 months, when HBsAg and HBeAg were detected, but anti-HBs was not tested. The boy suffered mild, acute HBV at the beginning of his second year. The peak alanine aminotransferase (ALT) concentration was 9.05 μkat/litre (the normal level of ALT is 0.15–0.90 μkat/litre). At the beginning of his 15th month, the ALT concentration had fallen to 2.15 μkat/litre. Normal ALT concentrations and seroconversion from HBeAg to anti-HBe were observed in his fourth year. The most recent examination of the boy was carried out at 6 years, when he was HBsAg positive, HBeAg negative, anti-HBe positive, and HBV DNA negative. Anti-HBs testing was never carried out.

HBV DNA encoding the "a" determinant and surrounding region of HBsAg was sequenced after amplification from the plasma of the child, taken at the end of his third year, and from his mother. The boy was infected with variants of HBsAg with predicted substitutions of arginine for the highly conserved cysteine at residue 137 or of serine for the highly conserved cysteine at residue 139 (Fig. 1). These substitutions were not seen in HBV DNA amplified from the mother but may have been present as minor populations. However, her virus had an unusual change predicted at residue 121, also affecting a conserved cysteine, accompanied by a further substitution at residue 120. HBV sequences from mother and son predicted an unusual substitution of arginine for threonine at residue 118 of HBsAg and seem to be of the *ayw* subtype.

Loss of the cysteines at residues 137 or 139 would be expected to alter the conformation and antigenicity of the "a" determinant, which depends upon disulphide bridging of cysteine residues. HBIG, given to neonates with the aim of neutralising maternal virus, occasionally may serve to select antibody escape variants present as minor populations of the mothers' virus pools [Zuckerman et al., 1994]. HBIG produced from plasma donated by individuals who have been immunised with HBV vaccine lacks the repertoire of antibody reactivity produced after natural infection and may be more likely to select such antibody escape variants [Lee et al., 1997]. Rarely, administration of HBIG derived from naturally immune donors, or from a mixture of naturally immune and immunised donors, may be insufficient to prevent transmission of HBV from viraemic mothers to their infants.

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